filed on February 8, 2002 (reference QX) for the date correction and see attached Exhibit B which shows that the word "pigs" should be replaced with the word "rats").

Claims 1, 19-20, 23, 25, 41, 60-62, 65, 67, 69, and 70 have been amended to delete the phrase "a biologically effective amount of." Claims 60-62, 65, 67, and 69 have been amended to replace the phrase "from which are derived omega-3 fatty acids selected from the group consisting of eicosapentaenoic acid, docosahexaneoic acid, and docosapentaenoic acid or a mixture thereof wherein the composition is administered for a time sufficient to increase the reproductive performance of the female swine" with the phrase "comprising C_{20} and C_{22} omega-3 fatty acids." Support for these claim amendments is found in original claim 7 and in Example 1.

I. <u>Applicants' claimed invention has met with great commercial success</u>.

If a product that embodies the invention supplants prior art products and is a great commercial success, then it can be inferred that the invention was not obvious because otherwise persons lured by the prospect of success would have developed the invention sooner. *Pentec, Inc. v. Graphic Controls Corp.*, 776 F.2d 309, 227 U.S.P.Q. 766 (Fed. Cir. 1985); *Cable Electric Products, Inc. v. Genmark, Inc.*, 770 F.2d 1015, 226 U.S.P.Q. 881 (Fed. Cir. 1985). In response to the Examiner's rejection, Applicants submit herewith, under 37 C.F.R. § 1.132, the declaration of Dr. Donald E. Orr, the President and Chief Operating Officer of United Feeds, Inc., the assignee of the captioned patent application, in which Dr. Orr describes in detail the great commercial success of the product that is the subject of Applicants' claimed method. The commercial success of the product that embodies the claimed method establishes that the claimed invention is nonobvious.

As Dr. Orr asserts in the attached § 1.132 declaration, the product (FERTILIUM™) that embodies the claimed method was introduced into the marketplace in February of 2002. FERTILIUM™ is an animal feed additive that contains marine animal

products, and is fed to female swine to increase the reproductive performance of female swine (*i.e.*, FERTILIUM™ is the product that embodies the method of the pending claims). Approximately 150,000 sows are already being fed FERTILIUM™, and current market analysis projections predict that FERTILIUM™ will be fed to approximately 200,000 sows by the end of 2002, 600,000 sows by 2003, and 1,000,000 sows by 2004. Approximately 130,000 pounds of FERTILIUM™ are currently being ordered per month from United Feeds, Inc., and, based on current market analysis projections, it is predicted that approximately 175,000 pounds will be ordered per month by the end of 2002, 525,000 pounds per month by 2003, and 875,000 pounds per month by 2004.

The commercial acceptance of FERTILIUM™ is directly related to the claimed invention (*i.e.*, a method of increasing the reproductive performance of female swine by administering a feed composition containing marine animal products to the female swine). The swine production business is very competitive and margins are very close. Swine producers have been impressed by the consistently good results obtained (*i.e.*, increased reproductive performance in sows) when sows are fed FERTILIUM™ and, thus, the effectiveness of FERTILIUM™ in increasing reproductive performance of sows has led to the commercial success of the claimed invention. The commercial success of FERTILIUM™ is evidenced in the attached § 1.132 declaration by the detailed sales and usage figures presented in the declaration, and, as Dr. Orr asserts in the attached declaration, the commercial success of FERTILIUM™ has been more rapid than expected based on his experience in new product development in the animal feed supplement market.

Furthermore, as Dr. Orr asserts in the attached § 1.132 declaration, FERTILIUM™ already has three to five times the market share that a flaxseed-containing product, that was on the market for a number of years before FERTILIUM™ was introduced, has for use in increasing reproductive performance in female swine, and FERTILIUM™ has only been on the market for about 8 months. Therefore, the great commercial success of

FERTILIUM™, the product that embodies the claimed method, and the short time within which FERTILIUM™ has supplanted prior art products in the same market (*i.e.*, the flaxseed-containing product) indicate that the claimed invention is nonobvious because otherwise persons lured by the prospect of commercial success would have developed the claimed invention sooner.

II. Rejection of claims 7, 60-62, 65, 67, and 69 under 35 U.S.C. § 103(a).

Flaxseed lacks C_{20} and C_{22} omega-3 fatty acids. *See* the § 1.132 declarations of Dr. Douglas M. Webel and Dr. Stephen K. Webel and Exhibit E transmitted herewith which show that flaxseed lacks C_{20} and C_{22} omega-3 fatty acids. Original claim 7 and amended claims 60-62, 65, 67, and 69 require that the feed composition administered to female swine contains marine animal products "comprising C_{20} and C_{22} omega-3 fatty acids." Accordingly, the subject matter of these claims cannot be obvious over U.S. Patent No. 5,110,592 (hereinafter the '592 patent) in view of Boudreaux et al. and statements on pages 1-3 of the specification because flaxseed, the feed additive described in the '592 patent, lacks C_{20} and C_{22} omega-3 fatty acids or esters thereof and original claim 7 and amended claims 60-62, 65, 67, and 69 require that the feed composition used in the claimed method contains C_{20} and C_{22} omega-3 fatty acids or esters thereof. Furthermore, none of Applicants' statements cited by the Examiner mention C_{20} and C_{22} omega-3 fatty acids; nor do they suggest that C_{20} and C_{22} omega-3 fatty acids are involved in female animal fertility (see discussion below, in particular, section V., part B.). Withdrawal of the rejection under 35 U.S.C. § 103(a) of claims 7, 60-62, 65, 67, and 69 is respectfully requested.

III. Rejection of claims 1-18, 41 (in part), and 69 under 35 U.S.C. § 112, second paragraph.

Claims 1-18, 41 (in part), and 69 stand rejected under 35 U.S.C. § 112, second paragraph, for indefiniteness. The Examiner indicates that the expression "reproductive

performance" is not defined in the claims and the specification. Applicants respectfully traverse the Examiner's rejection and contend that the expression "reproductive performance" is definite because "reproductive performance" is defined in the specification, and because the definition of "reproductive performance" was well-known in the art at the time of filing the captioned application.

Claims 1-18, 41, and 69 are directed to "a method of increasing the reproductive performance of a female swine." As stated on page 8, lines 6-14 of the specification, "[t]he reproductive performance of female animals may be increased by 1) increasing the number of live births to the female animal, 2) increasing the total births (*i.e.*, live and dead offspring) to the female animal, 3) decreasing the interval from weaning to estrus (*i.e.*, estrus is the period during which the female animal is capable of conceiving) for a female swine, 4) increasing the uniformity of birth weight of offspring of a female swine, 5) decreasing pre-weaning death loss of the offspring of a female swine, and 6) increasing the farrowing rate (*i.e.*, the percentage of animals that give birth) for female swine." Thus, reproductive performance is defined as encompassing the number of live births to a female swine, the number of total births to a female swine, the interval from weaning to estrus, the uniformity of birth weight, pre-weaning death loss of offspring, and farrowing rate. Accordingly, Applicants have clearly defined in the specification what is meant by "reproductive performance." Furthermore, as asserted by Dr. Douglas Webel and Dr. Stephen Webel in the attached § 1.132 declarations, the definition of "reproductive performance" was well-known in the art at the time of filing the captioned application. Withdrawal of the rejection of claims 1-18, 41, and 69 under 35 U.S.C. § 112, second paragraph, is respectfully requested.

IV. Rejection of claims 1, 20, 25, 41, 61-62, 67, 69, and 70 under 35 U.S.C. § 112, second paragraph.

Claims 1, 20, 25, 41, 61-62, 67, 69, and 70 stand rejected under 35 U.S.C. § 112, second paragraph, for indefiniteness. The Examiner indicates that the phrase "a biologically effective amount of" is indefinite. The phrase "a biologically effective amount of' has been deleted from claims 1, 20, 25, 41, 61-62, 67, 69, and 70 obviating the Examiner's rejection. Withdrawal of the rejection of these claims under 35 U.S.C. § 112, second paragraph, is respectfully requested.

V. Rejection of claims 1-20, 23, 25, 41, 60-62, 65, 67, 69, and 70 under 35 U.S.C. § 103 (a).

Claims 1-20, 23, 25, 41, 60-62, 65, 67, 69, and 70 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over the '592 patent in view of Boudreaux et al. and statements on pages 1-3 of the specification. The Examiner indicates that the '592 patent discloses that omega-3 fatty acids such as alpha-linolenic acid, eicosopentenoic acid, and docosohexanoic acid in an edible composition comprising flaxseed to be administered daily are useful in a method for increasing the number of live births to a female animal such as a female swine. The Examiner also states that the '592 patent teaches that flaxseed is known to contain omega-3 fatty acids such as alpha-linoleic acid, eicosopentenoic acid, and docosohexanoic acid, but that the '592 patent does not disclose the ratio of omega-6 fatty acids to omega-3 fatty acids in the composition. The Examiner indicates that Boudreaux et al. discloses a ratio of omega-6 to omega-3 fatty acids that is within the instant claims.

The Examiner further indicates that the Applicants teach on pages 1-3 of the specification that 1.) omega-3 fatty acids such as eicosopentenoic acid and docosohexanoic acid and docosapentaenoic acid are well known to be derived from fish oils and marine algae (page 2, lines 13-14), 2.) omega-6 fatty acids are known to increase the number of live births in animals (page 2, lines 24-25), 3.) salmon oil is known to be used in animal food (page 2,

lines 26-27), 4.) omega-3 fatty acids in particular are known to be useful to increase female animal fertility (page 2, lines 29-30), and 5.) salmon oil is known to contain both omega-3 and omega-6 fatty acids (page 3, lines 1-3).

The Examiner contends that, based upon all of the above-described teachings, it would have been obvious to a person of ordinary skill in the art at the time the invention was made to employ omega-3 and omega-6 fatty acids derived from fish oil in Applicants' claimed method for increasing the reproductive performance of female swine and to optimize the ratio of these fatty acids in the composition. Applicants respectfully traverse the Examiner's rejection. The invention of claims 1-20, 23, 25, 41, 60-62, 65, 67, 69, and 70 is not obvious over the '592 patent in view of Boudreaux et al. and statements on pages 1-3 of Applicants' specification. In response to the Examiner's rejection, Applicants also submit herewith, under 37 C.F.R. § 1.132, the declarations of Dr. Douglas M. Webel and Dr. Stephen K. Webel in which the declarants make factual statements in support of the arguments discussed below.

A. <u>The '592 patent teaches that flaxseed increases the reproductive performance of female swine</u>.

The claims of the instant application are directed to a method of increasing the reproductive performance of a female swine by administering to the female swine a feed composition comprising marine animal products. The Examiner contends that the '592 patent teaches that omega-3 fatty acids in an edible composition comprising flaxseed are useful in a method for increasing the number of live births in female swine. Contrary to the Examiner's contention, the '592 patent does not teach that <u>omega-3 fatty acids</u> increase the number of live births in female swine when fed to the animal in a feed composition. The '592 patent teaches that <u>flaxseed</u> increases the number of live births in female swine when included in a feed composition.

In this regard, the '592 patent describes administering to female swine a feed composition comprising ground flaxseed to increase the number of live births in female swine. The Applicant of the '592 patent merely speculates that the omega-3 fatty acid, linolenic acid, might be a component in flaxseed that increases the fertility of female animals, but states that other unknown compounds may be responsible for this effect. For example, in column 3, lines 10-24, of the '592 patent specification, the Applicant states that "*[w]hile not wanting to be bound by the following explanation,* it also *appears* that the linolenic acid in the flaxseed improves the fertility of the animal . . . flaxseed, however, may contain *other compounds which affect immunity and fertility but may be unknown at this time.*" (emphasis added). Accordingly, the Applicant of the '592 patent merely speculates that omega-3 fatty acids in flaxseed might increase the fertility of female swine, and clearly indicates that there are other unknown components in flaxseed that may be responsible for this effect.

Therefore, as Dr. Douglas Webel and Dr. Stephen Webel assert in the attached § 1.132 declarations, the '592 patent teaches that flaxseed, not omega-3 fatty acids, causes an increase in live births in female swine. Thus, the method of claims 1-20, 23, 25, 41, 60-62, 65, 67, 69, and 70 is not obvious over the '592 patent simply because marine animal products, as specified in Applicants' claims, contain omega-3 fatty acids. The teaching of the '592 patent that flaxseed causes an increase in live births in female swine does not render obvious a method of increasing the reproductive performance of female swine by administering a composition comprising marine animal products to female swine.

Furthermore, as discussed above, a flaxseed-containing product has been on the market as an animal feed additive for a number of years, and FERTILIUM™ has supplanted the flaxseed-containing product for use in increasing reproductive performance in female swine in the short time that FERTILIUM™ has been on the market. Thus, the commercial success of FERTILIUM™ over the flaxseed-containing product establishes that the claimed invention is nonobvious over the subject matter of the '592 patent because

otherwise people lured by the prospect of commercial success would have developed the claimed invention sooner.

Lastly, with respect to original claim 7 and amended claims 60-62, 65, 67, and 69, these claims require that a feed composition comprising marine animal products containing "C_{20} and C_{22} omega-3 fatty acids or esters thereof" be administered to the female swine. As Dr. Douglas Webel and Dr. Stephen Webel assert in the attached § 1.132 declaration, flaxseed lacks C_{20} and C_{22} omega-3 fatty acids or esters thereof. Accordingly, because flaxseed lacks C_{20} and C_{22} omega-3 fatty acids or esters thereof and claims 7, 60-62, 65, 67, and 69 require that the feed composition contains C_{20} and C_{22} omega-3 fatty acids or esters thereof, the subject matter of original claim 7 and amended claims 60-62, 65, 67, and 69 cannot be obvious over the '592 patent alone or in combination with any of Applicants' statements and Boudreaux et al.

B. Claims 1-20, 23, 25, 41, 60-62, 65, 67, 69, and 70 are not obvious over the '592 patent in view of Applicants' statement 4.

The Examiner further indicates that the Applicants teach on pages 1-3 of the specification that 1.) omega-3 fatty acids such as eicosopentenoic acid and docosohexanoic acid and docosapentaenoic acid are well known to be derived from fish oils and marine algae (page 2, lines 13-14), 2.) omega-6 fatty acids are known to increase the number of live births in animals (page 2, lines 24-25), 3.) salmon oil is known to be used in animal food (page 2, lines 26-27), 4.) omega-3 fatty acids in particular are known to be useful to increase female animal fertility (page 2, lines 29-30), and 5.) salmon oil is known to contain both omega-3 and omega-6 fatty acids (page 3, lines 1-3). Thus, the Examiner contends that claims 1-20, 23, 25, 41, 60-62, 65, 67, 69, and 70 are obvious over the '592 patent in view of Boudreaux et al. and the above-described statements.

The Examiner asserts that the Applicants state on page 2, lines 29-30, of the specification that "omega-3 fatty acids in particular are known to be useful to increase female animal fertility" (statement 4 above) and that claims 1-20, 23, 25, 41, 60-62, 65, 67, 69, and 70 are obvious over the '592 patent in view of this statement and Boudreaux et al. Contrary to the Examiner's assertion, the Applicants state on page 2, lines 29-30, that "the effects of linseed oil, and omega-3 fatty acids in particular, on increased sperm fertility and female fertility, applicable to cattle, sheep, and rats, have been studied (Abayasekara, *et al.*, 1999)." Respectfully, the Examiner has misquoted the Applicants' statement. Furthermore, when taken in the context of the Abayasekara et al. reference (cited by the Applicants in reference to this statement), Applicants' statement does not mean that "omega-3 fatty acids in particular are known to be useful to increase female animal fertility," but rather means that the effects of omega-3 fatty acids on fertility in cattle, sheep, and rats have been studied.

In Abayasekara et al. (attached as Exhibit A), the effects of polyunsaturated fatty acids (PUFAs; *e.g.*, omega-3 and omega-6 fatty acids) on fertility were studied. The authors first studied the effects of PUFAs on the types of prostaglandins and eicosanoids synthesized in response to feeding animals PUFAs because there are changes in prostaglandin and eicosanoid synthesis in response to administration of PUFAs to animals and these changes may be relevant to fertility. The authors emphasize that the changes in prostaglandin and eicosanoid synthesis are unpredictable in response to the administration of PUFAs to animals. For example, the authors tested the effects of α-linolenic acid on prostaglandin synthesis in animals. The authors make the following statement about these studies on page 279, column 1:

> In contrast, this dietary regime was also associated with changes in tissue phospholipid levels of PUFAs: increases in linoleic (18:2n-6), gamma linolenic (18:3n-6) and dihomogamma linolenic (20:3n-6) acids and a decrease in AA. The changes are not as expected and reinforce the need for experimental verification through monitoring of PUFAs and prostaglandin generation in the tissue(s) of interest in animals exposed to different PUFA diets.

The authors further studied the effects of dietary manipulations of PUFAs on eiconsanoid production and state that "[i]t is, therefore, clear that dietary manipulations of PUFAs can have major effects on eicosanoid production, although these are hard to predict." See page 279, column 2. Therefore, Abayasekara et al. teaches that the effects of dietary PUFAs on prostaglandin and eicosanoid synthesis are unpredictable.

Furthermore, the authors of Abayasekara et al. indicate that the effects of dietary PUFAs, including omega-3 and omega-6 fatty acids, on fertility are unpredictable. For example, the authors state on page 280, column 1, paragraph 2, that "[a]nimals on a high n-3 diet had increased ovulations in comparison with rats on a control diet whereas a diet high in n-6 PUFAs caused a decrease in the number of ova released." On page 281, column 2, paragraph 1, the authors contrast the effects on fertility of feeding cattle a diet supplemented with tallow versus yellow grease. The authors state that:

> Abomasal infusion of cattle with tallow (high in PUFAs but with only 2% linoleic acid) increased plasma PGFM ($PGF_{2\alpha}$) concentrations in response to an oxytocin injection in comparison to treatment with yellow grease containing 20% linoleic acid. Yellow grease in fact impaired the ability of the uterus to secrete $PGF_{2\alpha}$ possibly through inhibition of cyclooxygenase.

The authors also state with respect to male animals (page 282, column 2, paragraph 2) that "n-3 PUFA (linolenic acid) supplementation in the diet caused a marked decrease in testicular size and loss of fertility, whereas n-6 PUFA (linoleic acid) supplementation had no effect on testis size or fertility." Moreover, the authors conclude (page 282, column 2, last paragraph) that "[o]ur relative lack of knowledge means that it is impossible to predict at present whether particular dietary manipulations, which may be desirable from a human health viewpoint, will enhance or reduce fertility. Therefore, it is essential that further research into this general area is carried out before any changes in feed in terms of PUFA composition, are implemented." Accordingly, Abayasekara et al. teaches that the effects of dietary

supplementation with PUFAs on prostaglandin and eicosanoid synthesis and on fertility are difficult to predict.

Therefore, Applicants' statement taken in the context of Abayasekara et al. cannot mean that omega-3 fatty acids are known to be useful to increase female animal fertility because Abayasekara et al. teaches that the effects of omega-3 and omega-6 fatty acids on female fertility are unpredictable. In this regard, as Dr. Douglas M. Webel and Dr. Stephen K. Webel assert in the attached § 1.132 declarations, the Applicants' statement that "the effects of linseed oil, and omega-3 fatty acids in particular, on increased sperm fertility and female fertility, applicable to cattle, sheep, and rats, have been studied" taken in the context of Abayasekara et al. does not mean, as the Examiner suggests, that "omega-3 fatty acids in particular are known to be useful to increase female animal fertility," but rather Applicants' statement means that the effects of omega-3 fatty acids on increased female fertility have been studied.

Accordingly, the teaching of the '592 patent that flaxseed causes an increase in live births in female swine in combination with Applicants' statement that "the effects of linseed oil, and omega-3 fatty acids in particular, on increased sperm fertility and female fertility, applicable to cattle, sheep, and rats, have been studied" does nothing to render obvious a method of increasing the reproductive performance of female swine by administering to the animals a feed composition comprising marine animal products even if marine animal products contain omega-3 fatty acids. Furthermore, in light of the teaching of Abayasekara et al. that the effects of omega-3 fatty acids on reproductive performance are unpredictable, it would not have been obvious to a skilled artisan that marine animal products would increase female reproductive performance even though marine animal products contain omega-3 fatty acids.

Lastly, with respect to rejected claims 7, 60-62, 65, 67, and 69, because flaxseed lacks C_{20} and C_{22} omega-3 fatty acids or esters thereof and claims 7, 60-62, 65, 67,

and 69 require that the feed composition contains C_{20} and C_{22} omega-3 fatty acids or esters thereof, the subject matter of claims 7, 60-62, 65, 67, and 69 cannot be obvious over the '592 patent alone or in combination with Applicants' statement. Applicants' statement that "the effects of linseed oil, and omega-3 fatty acids in particular, on increased sperm fertility and female fertility, applicable to cattle, sheep, and rats, have been studied" does nothing to overcome the insufficiencies of the '592 patent with respect to claims 7, 60-62, 65, 67, and 69.

C. Claims 1-20, 23, 25, 41, 60-62, 65, 67, 69, and 70 are not obvious over the '592 patent in view of Applicants' statement 2.

The Examiner also asserts that the Applicants state on page 2, lines 24-25, of the specification that "omega-6 fatty acids are known to increase the number of live births in animals" (statement 2 above). Contrary to the Examiner's assertion, the Applicants state on page 2, lines 24-25, of the amended specification that "linseed oil and corn oil have been used in animal feed as a source of omega-6 fatty acids to increase the number of live births and to increase the number of weaned rats (Quackenbush, *et al.*, 1942)." Again, when this statement is taken in the context of Quakenbush et al., the statement does not mean that "omega-6 fatty acids are known to increase the number of live births in animals" as the Examiner suggests.

In Quackenbush et al. (attached as Exhibit B) two different diets were fed to rats and the effect of these diets on reproductive performance was determined. The diets were a "rice-extract diet" and a "yeast diet." These diets contained a coconut oil supplement (see page 1), a substantial lipid content derived from the rice extract and the yeast composition (see Table 2), and each of the diets was also supplemented with ethyl linolate or ethyl linolenate. Thus, the rats were not fed a diet containing only omega-6 fatty acids, but were fed omega-6 fatty acids in combination with many other lipids. Accordingly, Quakenbush et al. does not show that omega-6 fatty acids increase the number of live births in rats (*i.e.*, a mixture of lipids was fed to the rats). Therefore, the statement that "linseed oil

and corn oil have been used in animal feed as a source of omega-6 fatty acids to increase the number of live births and to increase the number of weaned rats" does not mean that omega-6 fatty acids alone are "known to increase the number of live births in animals" as the Examiner suggests.

Accordingly, the teaching of the '592 patent that flaxseed causes an increase in live births in female swine in combination with Applicants' statement taken in the context of Quakenbush et al. does not render obvious the method of claims 1-20, 23, 25, 41, 60-62, 65, 67, 69, and 70. As Dr. Douglas Webel and Dr. Stephen Webel assert in the attached § 1.132 declarations, Applicants' statement does not suggest that omega-6 fatty acids alone increase female fertility so the combination of the '592 patent and Applicants' statement does nothing to suggest that marine animal products would increase female reproductive performance even if marine animal products contain omega-6 fatty acids. Furthermore, in light of the teaching of Abayasekara et al. that the effects of omega-6 fatty acids on reproductive performance are unpredictable, it would not have been obvious to a skilled artisan that marine animal products would increase female reproductive performance even though marine animal products contain omega-6 fatty acids. Moreover, rats were used in the studies described in Quakenbush et al., and, accordingly, these studies do not render obvious Applicants' method of increasing the reproductive performance of female swine by administering marine animal products to the female swine. Thus, a method of increasing the reproductive performance of female swine by administering to the animals a feed composition comprising marine animal products is not obvious over the '592 patent in combination with Applicants' statement.

Lastly, with respect to rejected claims 7, 60-62, 65, 67, and 69, because flaxseed lacks C_{20} and C_{22} omega-3 fatty acids or esters thereof and claims 7, 60-62, 65, 67, and 69 require that the feed composition contains C_{20} and C_{22} omega-3 fatty acids or esters thereof, the subject matter of claims 7, 60-62, 65, 67, and 69 cannot be obvious over the '592

patent alone or in combination with Applicants' statement. Applicants' statement in reference to omega-6 fatty acids that "linseed oil and corn oil have been used in animal feed as a source of omega-6 fatty acids to increase the number of live births and to increase the number of weaned rats" does nothing to overcome the insufficiencies of the '592 patent with respect to claims 7, 60-62, 65, 67, and 69.

D. Claims 1-20, 23, 25, 41, 60-62, 65, 67, 69, and 70 are not obvious over the '592 patent in view of Applicants' statements 1, 3, and 5.

The Examiner also indicates that Applicants' statements that fish oils and marine algae contain omega-3 fatty acids (statement 1 above), that salmon oil contains both omega-3 and omega-6 fatty acids (statement 5 above), and that salmon oil is known to be used in animal food (statement 3 above) in combination with the '592 patent render obvious the method of claims 1-20, 23, 25, 41, 60-62, 65, 67, 69, and 70. In light of the teachings of Abayasekara et al. that the effects of omega-3 and omega-6 fatty acids on reproductive performance are unpredictable, Applicants' statements that fish oils and marine algae contain omega-3 fatty acids and that salmon oil contains omega-3 and omega-6 fatty acids do nothing to indicate that marine animal products would increase the reproductive performance of female swine. Applicants' statement that salmon oil is known to be used in animal food does nothing more to indicate that marine animal products would increase the reproductive performance of female swine. Thus, the teaching of the '592 patent that flaxseed increases reproductive performance of female swine in combination with Applicants' statement 1, 3, or 5 does not render obvious the method of claims 1-20, 23, 25, 41, 60-62, 65, 67, 69, and 70.

Moreover, with respect to rejected claims 7, 60-62, 65, 67, and 69, because flaxseed lacks C_{20} and C_{22} omega-3 fatty acids or esters thereof and claims 7, 60-62, 65, 67, and 69 require that the feed composition contains C_{20} and C_{22} omega-3 fatty acids or esters thereof, the subject matter of claims 7, 60-62, 65, 67, and 69 cannot be obvious over the '592 patent alone or in combination with Applicants' statements. Applicants' statements 1, 3, and

5 do nothing to overcome the insufficiencies of the '592 patent with respect to claims 7, 60-62, 65, 67, and 69.

E. Boudreaux et al. does nothing to overcome the insufficiencies of the '592 patent and of Applicants' statements.

The Examiner further indicates that Boudreaux et al. discloses a ratio of omega-6 to omega-3 fatty acids that is within the instant claims. Boudreaux et al. does nothing to overcome the above-described insufficiencies of the '592 patent and of Applicants' statements cited by the Examiner.

F. Summary

In sum,

1. The '592 patent teaches that flaxseed, not omega-3 fatty acids, causes an increase in live births in female swine.

2. The Applicants' statement 4 that "the effects of linseed oil, and omega-3 fatty acids in particular, on increased sperm fertility and female fertility, applicable to cattle, sheep, and rats, have been studied" taken in the context of Abayasekara et al. does not mean, as the Examiner suggests, that "omega-3 fatty acids in particular are known to be useful to increase female animal fertility," but rather Applicants' statement means that the effects of omega-3 fatty acids on increased female fertility have been studied.

3. The Applicants' statement 2 taken in the context of Quakenbush et al. does not suggest that omega-6 fatty acids alone increase female fertility. Furthermore, statement 2 is directed to Quakenbush et al. which describes studies in which the reproductive performance of rats was increased.

4. Accordingly, the teaching of the '592 patent that flaxseed causes an increase in live births in female swine in combination with Applicants' statement 2 or 4

does nothing to render obvious a method of increasing the reproductive performance of female swine by administering to the animals a feed composition comprising marine animal products even if marine animal products contain omega-3 and omega-6 fatty acids.

5. Furthermore, in light of the teaching of Abayasekara et al. that the effects of omega-3 and omega-6 fatty acids on reproductive performance are unpredictable, it would not have been obvious to a skilled artisan that marine animal products would increase female reproductive performance even though marine animal products contain omega-3 and omega-6 fatty acids.

6. In view of the teaching of Abayasekara et al. that the effects of omega-3 and omega-6 fatty acids on reproductive performance are unpredictable, Applicants' statements that fish oils and marine algae contain omega-3 fatty acids (statement 1), that salmon oil contains both omega-3 and omega-6 fatty acids (statement 5), and that salmon oil is known to be used in animal food (statement 3) do nothing in combination with the '592 patent to render obvious Applicants' claimed method.

7. Boudreaux et al. does nothing to overcome the above-described insufficiencies of the '592 patent and of Applicants' statements cited by the Examiner.

8. With respect to claims 7, 60-62, 65, 67, and 69, these claims cannot be said to be obvious over the '592 patent alone or in combination with Applicants' statements and Boudreaux et al. because flaxseed lacks C_{20} and C_{22} omega-3 fatty acids or esters thereof and claims 7, 60-62, 65, 67, and 69 require that the feed composition contains C_{20} and C_{22} omega-3 fatty acids or esters thereof.

Based on all of the above arguments (see also the accompanying § 1.132 declaration), withdrawal of the rejection of claims 1-20, 23, 25, 41, 60-62, 65, 67, 69, and 70 under 35 U.S.C. § 103(a) is respectfully requested.

CONCLUSION

The foregoing amendments and remarks are believed to fully respond to the Examiner's rejection. The amended claims are in condition for allowance. Applicants respectfully request allowance of the claims, and passage of the application to issuance.

Respectfully submitted,

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Appendix A to Amendment
Marked-Up Version of Replacement Paragraph(s) Under 37 C.F.R. § 1.121(b)(1)(iii)
Application No. 09/870,899

In the Background of the Invention section please replace the second paragraph on page 2 with the following paragraph:

-- Various oils have been used as sources of omega-3 and omega-6 fatty acids in animal feed. The lactational responses of dairy cows fed unsaturated fat from extruded soybeans or sunflower seeds have been studied (Schingoethe, *et al.*, 1996); flaxseed oil has been used in animal feed to increase the number of live births in sows, to increase the number of live weaned pigs, and to allow for earlier breeding (U.S. Pat. No. 5,110,592); conjugated linoleic acid has been used in animal feed to increase fat firmness, shelf life, and meat quality (U.S. Pat. No. 6,060,087); linseed oil and corn oil have been used in animal feed as a source of omega-6 fatty acids to increase the number of live births and to increase the number of weaned rats [pigs] (Quackenbush, *et al.*,[1941] 1942); salmon oil has been used in pet food to reduce damage to skin and mucosa in animals, such as dogs and cats, where the animal is afflicted with cancer and is subjected to radiation therapy (U.S. Pat. No. 6,015,798); the effects of linseed oil, and omega-3 fatty acids in particular, on increased sperm fertility and female fertility, applicable to cattle, sheep, and rats, has been studied (Abayasekara, *et al.*, 1999); modified tall oil supplemented swine animal feed has been used to improve the carcass characteristics of swine and to increase daily weight gain (U.S. Pat. No. 6,020,377); the use of salmon oil to increase sperm fertility in roosters using a 1.5:1 ratio of omega-6 fatty acids to omega-3 fatty acids has been studied (Blesbois, *et al.*, 1997), and the effect of dietary fatty acids on lactic acid bacteria associated with the epithelial mucosa has been studied (Ringo, *et al.*,1998).--

Appendix to B Amendment
Marked-Up Version of Rewritten Claims Under 37 C.F.R. § 1.121(c)(1)(ii)
Application No. 09/870,899

1. (Amended) A method of increasing the reproductive performance of a female swine, comprising the step of administering to the female swine [a biologically effective amount of] a feed composition comprising marine animal products containing omega-3 fatty acids or esters thereof that serve as a source of metabolites in the female swine to improve reproductive performance of the female swine.

19. (Amended) A method of increasing the number of live births to a female swine, comprising the step of administering to the female swine [a biologically effective amount of] a feed composition comprising marine animal products containing omega-3 fatty acids or esters thereof that serve as a source of metabolites in the female swine to increase the number of live births to the female swine.

20. (Amended) A method of increasing the total number of births to a female swine, comprising the step of administering to the female swine [a biologically effective amount of] a feed composition comprising marine animal products containing omega-3 fatty acids or esters thereof that serve as a source of metabolites in the female swine to increase the total number of births to the female swine.

23. (Amended) A method of increasing the uniformity of birth weight of offspring of a female swine, comprising the step of administering to the female animal [a biologically effective amount of] a feed composition comprising marine animal products containing omega-3 fatty acids or esters thereof that serve as a source of metabolites in the female swine to increase the uniformity of birth weight of offspring of a female swine.

25. (Amended) A method of increasing the farrowing rate of a female swine, comprising the step of administering to the female swine [a biologically effective amount of] a feed composition comprising marine animal products containing omega-3 fatty

acids or esters thereof that serve as a source of metabolites in the female swine to increase the farrowing rate of the female swine.

41. (Amended) A method of increasing the reproductive performance of a breeding population of swine comprising the steps of:

administering to a female swine [a biologically effective amount of] a feed composition comprising marine animal products containing omega-3 fatty acids or esters thereof that serve as a source of metabolites in the female swine to improve reproductive performance of the female swine; and

administering to a male swine [a biologically effective amount of] a feed composition comprising an oil containing omega-3 fatty acids or esters thereof that serve as a source of metabolites in the male swine to increase fertility of the male swine.

60. (Amended) A method of increasing the reproductive performance of a female swine, comprising the step of administering to the female swine [a biologically effective amount of] a feed composition comprising marine animal products [from which are derived omega-3 fatty acids selected from the group consisting of eicosapentaenoic acid, docosahexaneoic acid, and docosapentaenoic acid or a mixture thereof wherein the composition is administered for a time sufficient to increase the reproductive performance of the female swine] comprising C_{20} and C_{22} omega-3 fatty acids or esters thereof.

61. (Amended) A method of increasing the number of live births to a female swine, comprising the step of administering to the female swine [a biologically effective amount of] a feed composition comprising marine animal products [from which are derived omega-3 fatty acids selected from the group consisting of eicosapentaenoic acid, docosahexaneoic acid, and docosapentaenoic acid or a mixture thereof wherein the composition is administered for a time sufficient to increase the number of live births to the female swine] comprising C_{20} and C_{22} omega-3 fatty acids or esters thereof.

62. (Amended) A method of increasing the number of total births to a female swine, comprising the step of administering to the female swine [a biologically effective amount of] a feed composition comprising marine animal products [from which are derived omega-3 fatty acids selected from the group consisting of eicosapentaenoic acid, docosahexaneoic acid, and docosapentaenoic acid or a mixture thereof wherein the composition is administered for a time sufficient to increase the number of total births to the female swine] comprising C_{20} and C_{22} omega-3 fatty acids or esters thereof.

65. (Amended) A method of increasing the uniformity of birth weight of offspring of a female swine, comprising the step of administering to the female swine [a biologically effective amount of] a feed composition comprising marine animal products [from which are derived omega-3 fatty acids selected from the group consisting of eicosapentaenoic acid, and docosahexaneoic acid, docosapentaenoic acid or a mixture thereof wherein the composition is administered for a time sufficient to increase the uniformity of birth weight of offspring of the female swine] comprising C_{20} and C_{22} omega-3 fatty acids or esters thereof.

67. (Amended) A method of increasing the farrowing rate of a female swine, comprising the step of administering to the female swine [a biologically effective amount of] a feed composition comprising marine animal products [from which are derived omega-3 fatty acids selected from the group consisting of eicosapentaenoic acid, docosahexaneoic acid, and docosapentaenoic acid or a mixture thereof wherein the composition is administered for a time sufficient to increase the farrowing rate of the female swine] comprising C_{20} and C_{22} omega-3 fatty acids or esters thereof.

69. (Amended) A method of increasing the reproductive performance of a breeding population of swine comprising the steps of:

administering to a female swine [a biologically effective amount of] a feed composition comprising marine animal products [from which are derived omega-3 fatty

acids selected from the group consisting of eicosapentaenoic acid, docosahexaneoic acid, and docosapentaenoic acid or a mixture thereof wherein the composition is administered for a time sufficient to increase the reproductive performance of the female swine] comprising C_{20} and C_{22} omega-3 fatty acids or esters thereof; and

administering to a male swine a feed composition comprising a biologically effective amount of an oil from which is derived omega-3 fatty acids selected from the group consisting of eicosapentaenoic acid, docosahexaneoic acid, and docosapentaenoic acid or a mixture thereof wherein the composition is administered for a time sufficient to increase the fertility of the male swine.

70. (Amended) A method of increasing the reproductive performance of a female swine, comprising the step of administering to the female swine [a biologically effective amount of] a feed composition comprising marine animal products containing omega-3 fatty acids or esters thereof.

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Effects of altering dietary fatty acid composition on prostaglandin synthesis and fertility

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Summary Several studies over the past 20 years have demonstrated that subjects on diets composed of substances with high levels of n-3 polyunsaturated fatty acids (PUFAs) (e.g. fish) have a decreased incidence of heart disease. On this basis, a recent report from the Department of Health has advised UK consumers to decrease the proportion of saturated as opposed to unsaturated fats in their diet and to increase the ratio of n-3 to n-6 PUFAs. This could be achieved by altering the amounts of these constituents in milk and meat. n-3 Fatty acids can most easily be added to animal feed as either fish oil or linseed oil and can be increased in the blood and milk of ruminants following protection to avoid hydrogenation in the rumen. In western countries the ratio of consumption of n-6 to n-3 PUFAs is greater than 10 and current evidence tends to suggest that a ratio nearer 5 would be more desirable and compatible with cardiovascular well being. As fertility in the UK dairy herd is already poor, it is important to establish whether alterations in dietary n-3 and n-6 PUFAs affects herd fertility before widespread changes in animal diets are recommended. Therefore, this review considers the role played by PUFAs and eicosanoids in fertility, with particular reference to the implications for farm livestock production.

The evidence reviewed shows that alteration of the concentration and ratio of n-6 and n-3 PUFAs in feeds can influence prostaglandin synthesis/metabolism in a number of mammalian systems. The changed patterns of prostaglandin synthesis can as a consequence, affect the diverse functions (e.g. hormone secretion) that are normally mediated via prostaglandins. Similarly, changes in prostaglandin synthesis effected through manipulation of PUFAs has a major bearing on fertility (as PGs affect many reproductive parameters, e.g. ovulation). Several studies in cattle and other mammals, show that feeding or infusing different types of fat with varying PUFA content to females can alter: the number and size of ovarian follicles, the ovulation rate, progesterone production by the corpus luteum, the timing of luteolysis and gestational length. In the male most recent work has focussed on sperm production and experiments in fowl have demonstrated clear effects of dietary PUFAs on both the sperm membrane phospholipid composition and on fertilizing ability. © 1999 Harcourt Publishers Ltd.

BACKGROUND

Numerous studies over the past few years have reported that consumption of a diet high in n-3 polyunsaturated fatty acids (PUFAs) is associated with a decreased incidence of cardiovascular disorders.[1–8] Based on this extensive body of evidence, the UK population has been advised to change their diet such that foods rich in n-3 PUFAs forms a larger component of the diet than at present.[9] In other words, to lower the overall ratio of n-6 PUFA to n-3 PUFA from greater than 10 at present to around 5, which is commensurate with cardiovascular well being.[4] This change could be achieved by modifying the amount of n-3 PUFA contained in the main foods (meat and milk) which comprise the current UK diet. Modifying the n-3 PUFA content of milk and meat could be realised through feeding domestic ruminants (e.g. cattle, sheep) diets rich in n-3 PUFAs. However it is important that any recommendations for change in herd diet does not compromise fertility, as the fertility of the UK dairy herd is already poor and declining at a rate of 1% every 3 years. This review therefore considers the role of

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Exhibit A

dietary PUFAs and their related eicosanoid products in regulating fertility with particular emphasis on farm livestock production.

INTRODUCTION

Fatty acids

Fatty acids occur mainly as esters in natural fats and oils. However, they also exist in non-esterified form as free fatty acids (a transport form found in plasma). Fatty acids that occur in natural fats are usually straight chain derivatives and contain an even number of carbon atoms. The chain may be saturated (containing no double bonds) or unsaturated (containing one or more double bonds). Unsaturated fatty acids may be further subdivided as monounsaturated (one double bond),. polyunsaturated (PUFAs, two or more double bonds) and eicosanoids.

Animal tissues can synthesize the oleic acid (18:1n-9) family of unsaturated fatty acids (Fig. 1). However linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3), the nutritionally essential fatty acids (EFAs), cannot be synthesized endogenously as the required desaturases are absent.[5,10] They therefore have to be provided in the diet. Mammals were first shown to have an absolute requirement for EFAs in 1929.[11,12] Feeding animals a fat-free diet induced a state of EFA deficiency and a variety of pathophysiologic effects were noted: dermatitis, reproductive inefficiency and papillary necrosis. This deficiency state is characterized by a decrease in n-6 and n-3 PUFAs and by an accumulation of n-9 fatty acids, notably 20:3 (termed Mead acid) which is not a constituent of normal tissues (Fig. 1). The ratio of Mead acid to arachidonic acid has been used to define the deficiency state, with a ratio of greater than 0.4 being considered to be consistent with fatty acid deficiency.[13] The body has different requirements for n-6 and n-3 PUFAs, as they are involved in several, yet varied essential functions. For instance, docosahexaenoic acid (DHA 22:6n-3) derived from linolenic acid (18:3n-3) is essential for brain development and visual function,[14] whereas arachidonic acid (AA 20:4n-6) derived by elongation and desaturation from linoleic acid (18:2n-6), is the precursor of eicosanoids and is essential for neonatal growth.[15]

Linoleic acid is the precursor for AA, the unsaturated fatty acid from which the biologically active eicosanoids are derived.[16] Consuming diets deficient in linoleic acid lead to a decrease in tissue and blood arachidonic acid,[1] which results in poorer growth. It is to be expected that a decrease in the precursor (linoleic acid) would lead to a decrease in the product formed (AA). However, abundance of the precursor (linoleic acid) does not necessarily reflect the amount of product (AA) in certain species, as linoleic acid is not efficiently desaturated and elongated to AA in humans.[17,18] Therefore, while linoleic acid (which is abundant in nearly all commonly available vegetable oils, e.g. corn, sunflower, safflower and rape seed oils)[8] is the precursor for AA, the majority of the AA needed by carnivores is obtained from animal products.[18] In herbivores such as cows, the AA needed is provided equally by: (1) dietary intake of linoleic acid and (2) de novo synthesis from acetate and β-hydroxybutyrate (via fatty acid synthetases).[19,20] PUFA deprivation causes a general decrease in levels of AA, although the effects on the phospholipid composition of different tissues can be quite diverse. For example, it was found that AA concentrations in liver lipids were depleted, those in renal cortical lipids were unchanged, while the AA content of heart lipids actually increased.[21] Moreover, three physiological functions (dermal integrity, renal function and parturition) appeared to have a greater dependence on n-6 PUFAs, since they were better maintained with n-6 fatty acids when compared to their maintenance with n-3 fatty acids.[12,22]

Like the AA of the n-6 PUFAs, the long chain (n-3) PUFAs, DHA (22:6n-3) and eicosapentaenoic acid (EPA 20:5n-3) are also essential for many bodily functions. They can be delivered directly from the diet or produced within the body from the precursor α-linolenic acid.[8] α-Linolenic acid is present in all green leaf vegetables as a component of chloroplast lipids, although these lipids constitute only a small fraction of green leaf biomass. Linseed oil is one of the few vegetable oils that contain high levels of α-linolenic acid but it also contains significant quantities of linoleic acid.[8] α-Linolenic acid is present in grass, although concentrations are reduced during silage making. Fish oils are also high in α-linolenic acid and currently offer the most readily available dietary source of DHA and EPA.[23]

An interesting difference between plants and animals with regard to synthesis of PUFAs concerns the desaturation of oleic acid (n-9) to linoleic acid (n-6) and α-linolenic acid (n-3). In plants, oleic acid can be converted to linoleic and linolenic acids via the appropriate desaturases, whilst in animals the absence of these desaturases prevents linoleic and linolenic acid from being formed. In all animals including humans, desaturation takes place in the direction of the carboxyl group. This means that in animals, interconversion of the families of PUFAs does not take place.[5,17]

The fatty acid composition of blood, tissue and milk in non-ruminants generally reflects the fatty acid content of the diet. In contrast, while the diet of ruminants contains predominantly unsaturated fatty acids, the fat content of blood, tissues and milk is highly saturated. This difference can be explained by the extensive biohydrogenation of unsaturated fatty acids, which occurs in the rumen, through the activity of rumen microorganism's.[24]

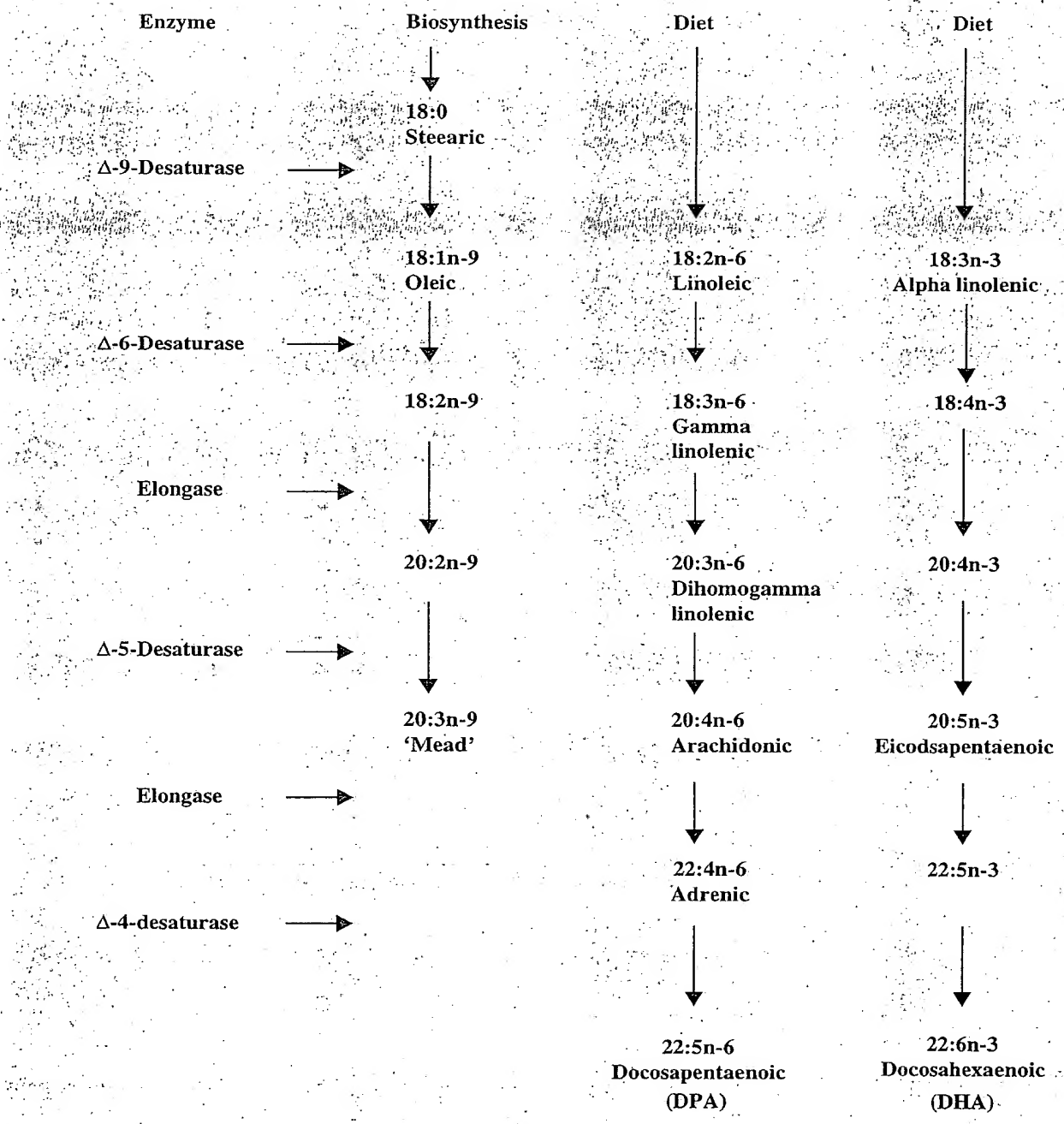

Fig. 1 Metabolic transformations of the three major unsaturated fatty acid families by desaturation and elongation.

Thus, in order to increase the supply of specific unsaturated fatty acids to the blood and tissues of ruminant animals, it is necessary to locate a dietary source rich in the chosen fatty acids and to protect the fatty acid from hydrogenation in the rumen. A number of techniques have been successfully developed to protect fats and oils which involve either chemical (e.g. formaldehyde or calcium salts) or physical (e.g. heat) treatment processes.[25,26]

Eicosanoids

The eicosanoids are derived from eicosanoic (C_{20}) fatty acids and comprise the prostaglandins, thromboxanes, leukotrienes and lipoxins.[13,27] As this review focuses on prostaglandins, they will be the only eicosanoids considered hereafter. Prostaglandins have been implicated in many reproductive functions and are reviewed below. They are also important for a variety of other physiological activities including controlling platelet aggregation and vascular homeostatis,[28] kidney function[27,29] inflammatory and immune responses,[30,32] hormone secretion (e.g. progesterone,[33] insulin)[34] and cell signalling.[27,35,36] In addition, eicosanoids are involved in the aetiology of several disease processes including hypertension[37] and tumour promotion.[38]

The most biologically active prostaglandins of the 2 series (dienoic prostaglandins) are derived from AA that has as its precursor linoleic acid.[5] In most cells AA is present in various cellular phospholipids in an esterified form and the generation of free AA is a rate-limiting step in eicosanoid synthesis[39] (Fig. 2). AA can be liberated from phospholipids directly via the action of an acyl hydrolase, phospholipase A_2(PLA_2)[40] or indirectly via the coordinated actions of phospholipase C (PLC) and diaclyglyceride lipase. The AA that is released via either of these two mechanisms is either immediately re-esterified or metabolized to: (1) prostaglandins and thromboxanes by prostaglandin synthetase (cyclooxygenase); (2) leukotrienes

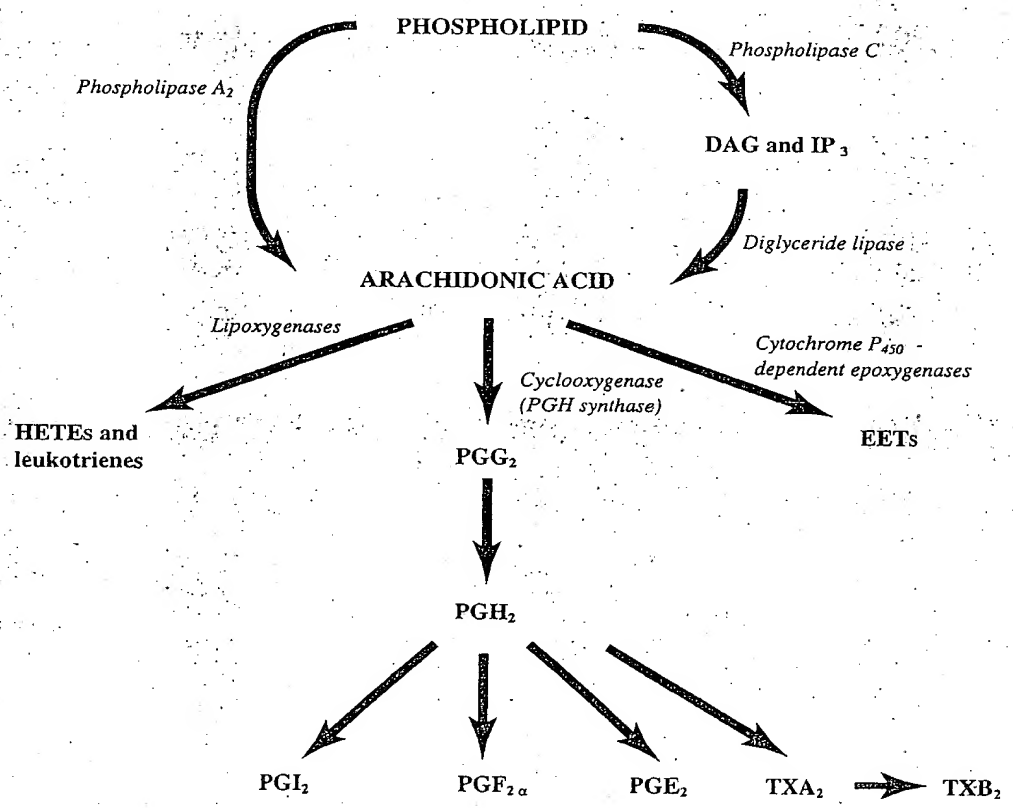

Fig. 2 Pathways of prostaglandin metabolism.

and hydroxy eicosatetraenoic acids (HETEs) by lipoxygenases; or (3) epoxyeicosatrienoic acids (EETs) by cytochrome P450-dependent epoxygenases.[13,27]

Trienoic prostaglandins (3 series prostaglandins) can be formed from α-linolenic acid (18:3n-3) which gives rise to PGE_3 and $PGF_{3\alpha}$. In contrast, the monoenoic prostaglandins (1 series prostaglandins) are derived from dihomogamma linolenic acid (20:3n-6) which gives rise to PGG_1, PGH_1, PGE_1 and $PGF_{1\alpha}$. In general, the 1 and 3 series prostaglandins are considered to be less biologically active than those of the 2 series.[6,19,41] However, as with all generalizations this too has exceptions. For an example, EPA-derived thromboxane A_3 TA_3 (as opposed to AA-derived TA_2)[42] is a weak aggregator of platelets,[43] whereas EPA-derived PGI_3 is as potent[41] as AA-derived PGI_2 as an antiaggregator.[44] Moreover, predicting the changes in the pattern of prostaglandin synthesis following dietary modulation of PUFAs is also difficult. For instance, when animals were fed diets rich in gamma linolenic acid (18:3n-6) supplemented with either EPA or DHA (both n-3), monoenoic prostaglandins were found to be increased in all tissues irrespective of the n-3 PUFA used to supplement the diet.[45] Since these animals lacked delta-5-desaturase (Fig. 1), this was a predictable outcome. In contrast, this dietary regime was also associated with changes in tissue phospholipid levels of PUFAs: increases in linoleic (18:2n-6), gamma linolenic (18:3n-6) and dihomogamma linolenic (20:3n-6) acids and a decrease in AA. The changes are not as expected and reinforce the need for experimental verification through monitoring of PUFAs and prostaglandin generation in the tissue(s) of interest in animals exposed to different PUFA diets. Nevertheless studies such as these clearly demonstrate the potential for modulating the generation of prostaglandins of altered potency by manipulating the PUFA composition of the diet.

EFFECTS OF DIETARY MANIPULATIONS OF ESSENTIAL FATTY ACIDS ON EICOSANOID SYNTHESIS

As eicosanoids have been implicated in numerous normal bodily functions and disease processes, much effort has been devoted to manipulating the dietary intake of PUFAs in a variety of species with a view to modulating synthesis of eicosanoids (review,[46] human,[5] cow,[47] pig,[48] rat,[49] salmon[50]). Three general experimental approaches have been utilized: (1) deprivation of PUFAs in the diet[29] (2) altering the ratio of n-3 to n-6 PUFAs in the diet,[45,47,49,51–57] and (3) altering the dietary intake of the eicosanoid precursor, AA itself.[18,58].

Feeding subjects diets rich in AA increased the plasma phospholipid levels of AA and the urinary excretion of the stable metabolites of prostacyclin (PGI_2) and TA_2.[59] Subjects fed diets rich in AA (n-6) and either DHA or EPA (both n-3), excreted greater quantities of PGI_2, but not TA_2 in urine.[59] In contrast, other studies involving murine macrophages have demonstrated that increased ingestion of n-3 PUFAs in the diet is associated with a decrease in generation of active prostanoids, i.e. prostaglandins of the 2-series.[60] This may be because the n-3 PUFAs replace AA in tissue phospholipids, which when released compete for prostaglandin synthase, and thereby competitively attenuate the rate of formation of 2-series prostaglandins (which are derived from n-6 PUFAs).[19,61] The direct inhibition of prostaglandin synthase activity by either high levels of n-3[62] or n-6 PUFAs[47] may also contribute to the decrease in overall prostaglandin generation. However, it has to be emphasized that caution should be exercised in extrapolating from animal studies into humans and vice versa as PUFAs (e.g. EPA) added in vitro or provided as a dietary supplement affects prostaglandin production differentially. For instance, human endothelial cells in vitro do not transform exogenous EPA into PGI_3,[63] whereas EPA provided as a dietary supplement increases excretion of PGI_3 in humans,[64] but not in rats.[65]

It is, therefore, clear that dietary manipulations of PUFAs can have major effects on eicosanoid production, although these are hard to predict. The relevance of this kind of experimental approach to probing the role of PUFAs in various processes associated with reproduction is considered next.

EFFECTS OF ALTERING DIETARY FATTY ACIDS ON FEMALE REPRODUCTION

Follicular development

The commencement of ovarian cyclicity heralded by the onset of puberty involves the recruitment, development and ovulation of follicles. Although several follicles are recruited at the start of an ovarian cycle, the number of follicles which go on to ovulate is characteristic for each species and ranges from one (e.g. man, cow) to several hundred (e.g. viscacha: *Lagostomus maximus*).[66] A number of studies in cattle have demonstrated that increasing the fat content of the diet increases both the number and size of follicles present in the ovary and in addition shortens the interval to the first ovulation post-partum.[67–73] The control diets employed indicated that it was the fatty acids themselves, rather than the additional energy they provided, which led to stimulation of the ovary. Two mechanisms of action have been suggested. The first is via increased blood cholesterol (both total and high density lipoprotein-cholesterol.[74] As cholesterol is the precursor of all steroids, increased substrate availability may increase follicular steroid synthesis. The following findings support this contention: (1) androstenedione lev-

els were found to be increased in follicular fluid of cows fed a high lipid diet and (2) granulosa cells obtained from follicles of cows fed a high-lipid diet increased progesterone output in vitro.[74] Oestradiol-17β produced by the coordinate actions of the steroidogenic enzymes in theca and granulosa cells, induces granulosa cell proliferation. This in turn would ultimately result in an increase in follicular size.

A second alternative, yet complementary, explanation could be that increased dietary fats led to an increase in AA in phospholipids of ovarian follicular granulosa cells. When released from phospholipids in response to gonadotrophin stimulation,[75] the AA could either have a direct effect on granulosa cells steroidogenesis[76,77] or be metabolized via the cyclooxygenase pathway to yield prostaglandins. These in turn may exert a stimulatory effect on granulosa cell steroidogenesis. The latter suggestion is supported by the observations that gonadotrophins stimulate prostaglandin production in ovarian follicular cells[78] and prostaglandins (PGE_2) in turn, are known to stimulate ovarian steroidogenesis.[79]

Ovulation

Follicular development culminates in the release of oocyte(s) at ovulation. In addition to effects on ovarian follicular steroid synthesis, AA and its metabolites have been implicated in ovulation in many mammalian species (e.g. rabbit,[80], pig,[81] rat,[82] rhesus monkey).[83] Follicular rupture is prevented by cyclooxygenase inhibitors.[83] This inhibitory effect can be overcome by administration of $PGF_{2\alpha}$.[83,84] Altering the dietary intake of n-3 and n-6 PUFAs led to considerable changes in ovulation rates in rats. Animals on a high n-3 diet had increased ovulations in comparison with rats on a control diet, whereas a diet high in n-6 PUFAs caused a decrease in the number of ova released.[49] Both diets caused an increase in PGE production, although the assay used did not distinguish between PGE_2 (derived from n-6 PUFAs, i.e. AA) and PGE_3 (derived from n-3 PUFAs). As high PGE levels are inhibitory, the authors suggest that the increased ovulation rate associated with the n-3 diet may have been due to a greater production of the less biologically active PGE_3 at the expense of the normal PGE_2. In contrast, the decrease in ovulation brought about by a high n-6 diet could be caused by increased production of PGE_2. While this is an attractive hypothesis confirmation will only be provided if: (1) it is clearly demonstrated that a high n-3 diet leads to a change in the ratio of PGE_3: PGE_2 and (2) PGE_2-induced decreases in ovulation can be blocked by PGE_2 receptor antagonists.

The cessation of ovulation associated with menopause is linked to an increase (~15%) in serum cholesterol.[85] The rise in serum cholesterol appears to be related to the dietary intake of PUFAs, as the elevation in plasma cholesterol in women on a high PUFA diet was less than that in women on a lower PUFA intake.[85] This finding offers the intriguing possibility of the existence of an inverse relationship between PUFA intake and plasma cholesterol levels. Unfortunately, this study did not define the type of PUFA (n-3 or n-6) whose intake was altered. Nevertheless, it does suggest that PUFAs affect ovulation, either by modulating levels of (1) prostaglandins and/or (2) serum cholesterol. However, it is unlikely that changes in serum cholesterol influence ovulation directly. Instead, it may be that changes in serum cholesterol reflects alterations in intakes of particular PUFAs as increased consumption of n-3 PUFA has been shown to cause an increase in cholesterol levels in hamsters.[86] The converse may also hold true, i.e. that lower serum cholesterol is associated with a low intake of n-3 PUFA which in turn could affect the ovulatory processes through modulating the generation of 2- and 3-series prostaglandins.

Corpus luteum function

Follicular rupture leads to the formation of the corpus luteum. Tha fat composition of the diet has been suggested to influence luteal function in three different ways: by a direct action on progesterone production; via alteration of the production of eicosanoids within luteal tissue and/or by interaction with the system controlling luteolysis and the maternal recognition of pregnancy.

Lipid infusion to either cattle or sheep during the luteal phase increases serum concentrations of progesterone.[87–90] As discussed previously, this may have been due to raised cholesterol levels providing an increase in available precursor for progesterone biosynthesis. Another possibility is that clearance rates of progesterone from plasma may be reduced.[89] Both soybean oil and olive oil increased circulating progesterone concentrations, although soybean oil was more effective.[90] This elevation in progesterone could have a beneficial effect on fertility, as sub-optimal progesterone concentrations are associated with high return rates in cows.

The corpus luteum also has the capability of producing a variety of eicosanoids, including $PGF_{2\alpha}$, PGE_2 and PGI_2 (prostacyclin) in addition to products of the lipoxygenase pathway, particularly 5-HETE.[91] $PGF_{2\alpha}$ has an inhibitory effect on progesterone production. This was first demonstrated for the sheep corpus luteum[92] and subsequent studies have extended these observations to most mammalian species.[93–97] In contrast, PGE_2 and PGI_2 are luteotrophic[79,93,98–100] and PGE_2 has been shown to influence its own secretion from bovine corpora lutea.[101] Altering the balance of endogenous prostaglandin production within the corpus luteum may therefore alter both the concentration of progesterone produced and the overall length of the luteal phase.

The increase in luteal $PGF_{2\alpha}$ at the time of luteal regression in rats may be due, in part, to an increase in PLA_2 activity.[102] An increase in PLA_2 activity leads to an increase in AA, and hence its metabolites including $PGF_{2\alpha}$ which are then available to exert an inhibitory action on progesterone production. The finding that the bovine corpus luteum contains very high levels of PUFAs, especially AA, esterified in phospholipids[103] supports this notion. Furthermore the levels of n-6 PUFAs (a source for AA) is highest in the mid- and late-luteal phase of corpora lutea of domestic ruminants (sheep,[104] cow[105]). Luteal cells from cows fed a diet high in n-3 PUFA produce significantly greater quantities of progesterone under basal conditions.[106] This suggests that products of n-3 PUFA oxygenation, namely the trienoic prostaglandins, are capable of stimulating progesterone production by luteal cells. Whilst no direct evidence for this contention exists in luteal tissue, the finding that PGE_3 stimulated testosterone synthesis by testis Leydig cells of goldfish, tends to support this hypothesis.[107]

Infusion of lipids to sheep increased serum concentrations of both the $PGF_{2\alpha}$ metabolite PGFM (13, 14-dihydro-15-keto $PGF_{2\alpha}$) and PGE_2. Olive oil was more effective than soybean oil in this respect and also caused a significant shortening of oestrous cycle length.[90] This difference was perhaps surprising as soybean oil contains more of both linoleic and linolenic acid than olive oil. However, linoleic and linolenic acid can also reduce both AA and prostaglandin synthesis via inhibition of the enzymes PLA_2 and cyclooxygenase.[47,108,109] Excess (n-3) or (n-6) PUFA concentrations may therefore down-regulate dienoic prostaglandin synthesis.

Luteolysis and the maternal recognition of pregnancy

As well as direct effects on the corpus luteum, PUFAs can influence luteal activity via interaction with the uterus. Luteal regression in domestic ruminants and pigs is caused by uterine secretion of $PGF_{2\alpha}$.[94,110,111] In cattle and sheep $PGF_{2\alpha}$ is released from the endometrium in response to oxytocin from the corpus luteum.[112] Oxytocin binding to its uterine receptor leads to the activation of both PI-PLC and PLA_2[113,114] which causes release of AA and its subsequent metabolism via cyclooxygenase to yield $PGF_{2\alpha}$. As in other tissues, availability of AA in the endometrium determines the ability of this tissue to synthesise prostaglandins. Addition of AA to bovine endometrial explants in vitro caused a dramatic increase in prostaglandin output.[115] However, the basal output of 2-series prostaglandins (PGE_2, $PGF_{2\alpha}$ and PGI_2) from endometrial explants in vitro of cows fed a diet high in n-6 PUFAs was decreased compared to controls whereas n-3 PUFA dietary supplementation had no effect on the production of dienoic prostaglandins.[116] This highlights the differences in outcome, in terms of prostaglandin output between PUFA addition in vitro and the dietary supplementation with PUFAs.

Abomasal infusion of cattle with tallow (high in PUFAs but with only 2% linoleic acid) increased plasma PGFM concentrations in response to an oxytocin injection in comparison to treatment with yellow grease containing 20% linoleic acid.[47] Yellow grease in fact impaired the ability of the uterus to secrete $PGF_{2\alpha}$, possibly through inhibition of cyclooxygenase. As higher concentrations of linoleic acid have been found naturally in the endometrium of pregnant versus non-pregnant cows,[117] it has been suggested that this may form part of the normal anti-luteolytic mechanism. During the establishment of pregnancy (maternal recognition of pregnancy) in sheep and cattle, the normal anti-luteolytic mechanism is mediated via the indirect actions of trophoblast interferon, IFNτ, which is thought to act principally by inhibiting the expression of endometrial oxytocin receptors.[118–120] IFNτ could also use alternative mechanisms to inhibit $PGF_{2\alpha}$ synthesis via alterations in lipid metabolism. It is possible that IFNτ redirects AA metabolism down the expoxygenase pathway, thereby decreasing the availability of AA for metabolism via the cycloxygenase pathway.[121] If this were the mechanism used, addition of IFNτ to endometrium either in vivo or in vitro should lead to a decrease in basal $PGF_{2\alpha}$ production. This has indeed been demonstrated.[122,123] Moreover, the possible molecular mechanism(s) utilized by IFNτ in directly inhibiting $PGF_{2\alpha}$ synthesis is provided by the findings that oxytocin-induced expression of cyclooxygenase-2 (the inducible form of the enzyme) and prostaglandin F synthase are inhibited by IFNτ in bovine endometrial cells.[124]

Parturition

The involvement of PUFAs in parturition was first demonstrated in rats the 1930s,[125] whereas rats fed on a diet free of EFAs were found to have a prolonged gestation period (by 1 to 3 days). Prostaglandins are key hormones both in terms of cervical ripening and myometrial contractility, which are essential for mammalian parturition.[126] Therefore, the connection can be made between changes in dietary intake of PUFAs and the ensuing changes in gestational length.

Changes in PUFA intake through altering the pattern of prostaglandin production may influence either the timing or efficiency of the onset of labour. Support for this assertion is provided by numerous studies in animals[125–130] as well as humans.[131–133] In general, animals or humans fed diets high in n-3 PUFAs exhibited an increase in gestational length. This has been attributed to the changed pattern of PG synthesis, which gives rise to an increase in the generation of 3-series prostaglandins. Since 3-series

prostaglandins are less potent than the 2-series prostaglandins (e.g. in terms of inducing contraction)[6,132] normally associated with parturition, the suggestion is that the biological activity of these 3-series prostaglandins is insufficient to induce the vigorous myometrial contractions associated with normal labour. That this may be the case is supported indirectly by the finding that rats fed a diet high in linolenic acid (n-3) were able to reproduce normally providing the pups were delivered by caesarean section.[130] The implication therefore is that n-3 PUFA derived 3-series prostaglandins are capable of substituting for n-6 PUFA derived 2-series prostaglandins in all reproductive processes with the exception of parturition.

Evidence exists to support the idea that normal onset of labour is associated with an increase in n-6 PUFA derived dienoic prostaglandins: (1) plasma levels of linoleic and arachidonic acid (both n-6) are higher than those of linolenic acid, EPA and DHA (all n-3) in women in labour;[134] (2) levels of arachidonic acid increased throughout pregnancy, with highest levels being observed during labour, followed by a rapid decline postpartum;[135] and (3) levels of linoleic acid (the precursor of arachidonic acid – Fig. 1) increased in uterine arteries during pregnancy.[136] From the foregoing it is clear that there exists a large body of evidence to support the idea that normal labour is associated with an increase in n-6 PUFA derived 2-series prostaglandins. It would also appear that an increase in intake of n-3 PUFA during gestation leads to the process of parturition being compromised due to an increase in the less potent 3-series prostaglandins and a concomitant decrease in 2-series prostaglandins. There is compelling evidence to support the latter notion: n-3 PUFAs added in vitro to human decidual cell cultures significantly decreased the production of the 2-series prostaglandins PGE_2 and $PGF_{2\alpha}$[137] and infusion of n-3 PUFAs in vivo into pregnant sheep caused a decrease in both maternal and foetal plasma levels of PGE_2.[128] However direct evidence to support the former is rather surprisingly lacking, i.e. no direct measurements of 3-series prostaglandins have been made following augmentation with dietary n-3 PUFAs. In our view these measurements need to be performed before the hypothesis that n-3 PUFAs prevents the normal onset of labour by giving rise to 3-series prostaglandins in the uterus can be accepted as fact.

Lactation

Parturition is followed by lactation. There are numerous studies showing that it is possible to manipulate the fatty acid composition of milk by feeding protected unsaturated fats and oils.[26,138,139] There is considerable interest in this work in relation to the production of milk with a higher unsaturated to saturated fat ratio to benefit human health. This topic merits a review in its own right and is not considered here.

MALE FERTILITY

Eicosanoids[140] and PUFAs have also been implicated in male reproductive function. AA itself, as well as prostaglandins and leukotrienes, have been implicated in mediating the stimulatory actions of luteinizing hormone on testicular steroid synthesis,[141–143] where AA release is effected through activation of PLA_2.[144]

n-3 PUFA (linolenic acid) supplementation in the diet caused a marked decrease in testicular size and loss of fertility, whereas n-6 PUFA (linoleic acid) supplementation had no effect on testis size or fertility.[145] The decrease in testicular size was found to be due to a degeneration of seminiferous tubules and loss of germ cells associated with an absence of spermatozoa. Interestingly, there was no change in Leydig cell number.[145] Addition of AA (n-6 PUFA) to Leydig cells caused an increase testosterone output[107] which was antagonised by EPA (n-3 PUFA) even though PGE_3 (a product of n-3 PUFA oxygenation) was found to be capable of stimulating steroid synthesis.[107]

In mammals, the lipid composition of sperm membranes plays a major role in the physicochemical modifications leading to fertilization.[146] In all species, phospholipids are the major lipid components of spermatozoa and they contain large amounts of PUFAs.[147–149] Fowl fed on diets containing different compositions of n-3 or n-6 PUFAs yielded sperm containing altered PUFAs in membranes. This suggested that transfer of PUFAs from diet to sperm is effective. High n-3 PUFA levels in the diet led to increases in n-3 PUFA in sperm membranes and this was associated with an increase in fertilizing ability and semen quality.[53,54]

CONCLUSIONS

The preceding survey has established that changes in dietary PUFA composition can affect membrane phospholipid PUFA content and alter prostaglandin synthesis. It has also illustrated the diverse and profound roles played by eicosanoids in general and prostaglandins in particular, in reproduction and fertility. Moreover, this survey has highlighted the paucity of information pertaining to lipid metabolism in endometrial and ovarian tissues of domestic ruminants during cyclicity or gestation. Our relative lack of knowledge means that it is impossible to predict at present whether particular dietary manipulations, which may be desirable from a human health viewpoint, will enhance or reduce fertility. Therefore, it is essential that further research into this general area is carried out before any changes in feed in terms of PUFA composition, are implemented as the fertility of the UK dairy herd is

already extremely poor with average conception rates of only around 50%.

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THE EFFECTIVENESS OF LINOLEIC, ARACHIDONIC, AND LINOLENIC ACIDS IN REPRODUCTION AND LACTATION [1, 2]

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In comparing individual fatty acids or their derivatives for biological activity, restoration of growth and prevention or cure of dermal lesions have served heretofore as the criteria (Burr and Burr, '30; Burr, Burr and Miller, '31; Turpeinen, '38; Hume et al., '40). Although reproduction is seriously impaired in fat deficiency, little is known concerning the unsaturated acid requirements for this important biological function. Normal litters of rats have been produced with dietary supplements of natural fats such as lard and butterfat (Burr and Burr, '30; Evans et al., '34; Maeder, '37), and inferior litters with linoleic acid either as a concentrate (Evans et al., '34), or as the pure methyl ester (Mackenzie et al., '39). Data concerning other acids or esters are lacking.

In experiments with low-fat diets most workers have used ether-extracted yeast as the source of the vitamin B complex (Burr and Burr, '30; Evans et al., '34). The use of this material is open to criticism since it has been shown that yeast contains from 2.5 to 4% of lipids which are firmly bound and non-extractable by ether (Smedley-Maclean, '22; Newman and Anderson, '33). Mackenzie, Mackenzie and McCollum

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Exhibit B

('39) have recently used a water-soluble extract from yeast, but unfortunately, they fed a relatively low level of methyl linolate.

Recent work by Schneider and Steenbock ('39) had shown that a commercially available water-soluble rice bran concentrate very low in lipids, constituted a good source of the vitamin B complex when fed in a diet which contained egg white as the source of protein. In the present work this product was fortified with riboflavin to make a satisfactory diet in which purified casein served as the protein. In parallel experiments using this diet of extremely low fat content and a diet containing ether extracted yeast, unsaturated acids of high purity were compared for their nutritive value.

EXPERIMENTAL

Diets

The first diet, designated the "Rice-Extract Diet," contained glucose [3] and a rice bran concentrate [4] with added riboflavin; the second, which was a duplication of the Burr and Burr diet, designated the "Yeast Diet," contained sucrose and ether-extracted yeast (table 1).

Both diets were supplemented with 100 μg. carotene, 35 μg. calciferol, 350 μg. tocopherol [5] and 100 μg. vitamin K_1 [5] per rat per week. These supplements were dissolved in a liquid fraction of hydrogenated coconut oil (I. V. = 0.1), and 1 drop of the solution was administered twice weekly. The flavin was dissolved in N/50 acetic acid, mixed with the rice bran concentrate and incorporated in the ration. The yeast was fed in small dishes.

Although the yeast had been extracted with ethyl ether in a continuous extractor for 3 days, the daily intake of unsaturated lipid from yeast was many times as high as that from the rice bran concentrate (table 2). This was revealed

[3] Cerelose.

[4] Vitab Type II, National Oil Products Company.

[5] We are indebted to Merck and Company, Inc. for the α-tocopherol and vitamin K_1.

by treatment of both desiccated and moist samples of the two materials with acidified alcohol and subsequent extraction of the products. In the first case 100 gm. of yeast or rice bran concentrate were dried over P_2O_5 and refluxed for 2 hours

TABLE 1

Composition of diets.

	RICE-EXTRACT DIET	YEAST DIET
Casein (alcohol-extracted)[1]	18	18
Wesson salts	4	4
Cerelose	75	..
Sucrose	..	72
Ether-extracted yeast[2]	..	0.65 gm./day
Rice bran concentrate[3] + 2 mg. riboflavin/kg. ration	3	..

[1] Labor for the purification of the casein was furnished by the Works Progress Administration.

[2] Brewers yeast, Anheuser-Busch Company.

[3] Vitab type II, National Oil Products Company.

TABLE 2

Lipid content of dietary constituents by cleavage with HCl.

CONSTITUENT	TOTAL LIPID (P. E. EXTRACT)	IODINE VALUE	APPPROXIMATE DAILY LIPID INTAKE[1]	
			Total	Unsaturated (calcd. as linoleic)
	%		*mg.*	*mg.*
Yeast (desiccated)	2.43	85.7	16.0	7.6
Rice bran concentrate (desiccated)	0.36	64.0	1.1	0.4
Yeast (moist)	4.21	84.7	27.0	12.6
Rice bran concentrate (moist)	0.44	41.6	1.3	0.3
Casein	0.11	40.4	2.0	0.4

[1] Based on an assumed consumption of 10 gm. of diet daily.

with 300 cc. of a solution of 3% dry HCl in absolute alcohol. The extract was diluted with an equal volume of water and shaken with three 100 cc. portions of chloroform. The alcohol-insoluble residue was extracted with chloroform for 2 days

in a Soxhlet. The chloroform extracts were combined, washed thoroughly with water, dried over sodium sulphate and freed from chloroform under reduced pressure. The residue was taken up in 5 to 10 cc. of chloroform and freed from non-lipid substances by precipitation with petroleum ether. The petroleum ether-soluble material was washed with water, dried and freed from solvent.

Hydrolysis with 3% HCl in 80% alcohol produced even a larger yield of lipid from the yeast. The procedure was identical with that detailed above except that before refluxing, 20% of water was added to the yeast to make it equivalent in water content to the rice bran concentrate.

The casein was purified by extraction with warm alcohol (50°C.). Nevertheless, it yielded a small amount of lipid on acid hydrolysis (table 2). For the hydrolysis 100 gm. were heated with 300 cc. of dilute HCl (1:3) for 20 hours on a steam bath. The solution was cooled, treated with an additional 25 cc. of HCl and extracted with three 100 cc. portions of redistilled petroleum ether. The combined extracts were washed with three 100 cc. portions of water and dried over sodium sulphate. The solvent was removed under reduced pressure and the residue weighed, giving a yield of 90 mg. When the aqueous phase was refluxed vigorously for 6 hours more, an additional 20 mg. of lipid were obtained giving a total of 110 mg.

Based on these analyses, the yeast furnished 27 mg. of lipid per rat daily. Assuming an average food intake of 10 gm. daily the rice bran concentrate furnished 1.3 mg. of lipid. In terms of unsaturated lipid these differences were even greater. Expressing the unsaturated compounds in terms of linoleic acid, a maximum of 12.6 mg. of linoleic acid was furnished by the yeast and 0.4 mg. by the rice bran concentrate. Adding the lipid which was supplied by the casein these values become 13.0 and 0.8 mg. for the yeast and rice-extract diets, respectively.

The fatty acids were fed as the ethyl esters. The ethyl linolate and linolenate were prepared from corn oil and

linseed oil by a modification of the Rollett method ('09). The ethyl arachidonate was prepared from beef suprarenal phosphatide [6] by low temperature crystallization and fractional distillation (Shinowara and Brown, '40). The distilled ethyl esters had the following iodine values (Wijs, 4 hr.): ethyl linolate 166 (theor. 164.8); ethyl linolenate 245 (theor. 248.9); ethyl arachidonate 299 (theor. 305.5).

Preparation of animals

To minimize the pre-experimental storage of essential unsaturated fatty acids, litters at 12 days of age were transferred with their mothers to a diet consisting largely of potatoes (Quackenbush et al., '39). The young were weaned when they weighed 40 gm. and placed in individual metal cages where they consumed the experimental diets ad libitum. Thirty female rats were given the rice-extract diet, and twelve females and fourteen males the yeast diet. After 12 weeks the females on each diet were divided into five groups. The heaviest animals, weighing about 178 gm., received only the basal diet; the lightest, which averaged 131 gm., received in addition supplements of cottonseed oil. The remainder were divided equally among the other groups. Three weeks later all were mated with normal males. After the finding of sperm in the vaginal smear the allowances of fat-soluble vitamins and vitamin B complex were doubled. The litters were reduced to six young each, 24 hours after parturition. During lactation the dose of the vitamin B complex was tripled.

RESULTS

Growth and general appearance

Animals fed the low-fat diets grew to maturity, but at the twelfth week their body weights were significantly less than those of comparable animals fed our stock diet (Steenbock, '23). Those getting the rice-extract diet weighed 151 gm. and

[6] We are indebted to Dr. O. Kamm of Parke-Davis and Company for this preparation.

those receiving the yeast diet 154 gm., while females in the stock colony weighed 195 gm. at the same age.

The addition of various lipid supplements after 12 weeks on either of the two basal diets resulted in growth, the magnitude of which depended upon the nature of the supplement as well as on the basal ration. In 3 weeks cottonseed oil produced a gain of 50 gm. in the animals ingesting the rice-extract diet and 30 gm. in those fed the yeast diet. Ethyl linolate produced a gain of 26 gm. on either diet, while ethyl arachidonate produced a gain of 34 gm. on the rice-extract diet and 19 gm. on the yeast diet. Ethyl linolenate produced much smaller gains, viz., 14 gm. on the rice-extract diet and only 8 gm. on the yeast diet. Without supplements the animals on the former diet gained slightly, viz., 7 gm.; those on the latter diet did not gain. A further demonstration of the necessity of fatty acids for growth was obtained from two groups of animals which had received ethyl linolate or cottonseed oil beginning with the second instead of the twelfth week on the rice-extract diet. Those receiving ethyl linolate weighed 188 gm. and those getting cottonseed oil weighed 195 gm. at the end of the twelfth week.

The animals on either of the low-fat diets developed a scaliness of the hind paws and tail after they had been on the experimental diet from 9 to 12 weeks. No scaliness or loss of hair around the eyes, nose or mouth was noted. Within 3 weeks after supplements of cottonseed oil, ethyl linolate or ethyl arachidonate were given, the scaliness of the hind paws and tail was cured completely. The cured animals were sleek and in every way appeared like stock rats. However, with ethyl linolenate the scaly condition of the hind paws and tail persisted even after 7 weeks.

Reproduction and lactation

A failure of normal parturition always ensued on the basal diets (table 3). Labor began at term, but parturition was accompanied by excessive hemorrhage and was not completed for 2 to 3 days. The animals lost weight and became extremely

weak and anemic. Two of the females died before giving birth to their young. All of the young died within 48 hours after birth.

When supplements of cottonseed oil, ethyl linolate, or ethyl arachidonate were fed, the needs of both parturition and lactation were met. The rice-extract diet gave approximately the same results as the yeast diet with the same fat supplements. When ethyl linolate was given after the second week of the experimental period, the results compared favorably with those obtained on our stock diet.

TABLE 3

The effects of fatty acid esters on reproduction.

GROUP	SUPPLEMENT	AMOUNT FED DAILY	RICE-EXTRACT DIET					YEAST DIET	
			No. of rats	Gestation period	Mean no. young per litter	Born alive	Weaned	Born alive	Weaned
	12th to 19th week:	*drops*		*days*		%	%	%	%
1	None	...	6	25	4	8	0	73	0
2	Ethyl linolate	5	5	22	6	100	34	100	83
3	Ethyl linolenate	5	5	24	3	56	0	83	0
4	Ethyl arachidonate	5	5	22	9	100	67	100	55
5	Cottonseed oil	10	2	22	5	100	80	100	92
	2nd to 19th week:								
6	Ethyl linolate	5	5	22	6	100	79	..	..
7	Cottonseed oil	5% in diet	2	22	6	100	89	..	..

By way of contrast ethyl linolenate produced results only slightly better than those obtained on the basal diet. While the gestation period was prolonged and vaginal bleeding was the same as on the basal diets, three of the fourteen young born alive in group 3, survived the first week. One of these reached a weight of 40 gm. at 5 weeks and the other two a weight of 31 gm. at 4 weeks of age, but all died shortly thereafter. All had scaly feet and tails, in contrast with the young receiving the other lipid supplements, which were entirely normal.

Other fat deficiency symptoms

Failure of oestrus as reported by Burr and Burr ('30) and Evans et al. ('34) was not observed in our animals during 20 weeks on low-fat diets. Pseudo pregnancies appeared in two of our deficient animals. This defect was not corrected by ethyl linolate. Resorptions as reported by Maeder ('37) were not observed in any of our animals.

A comparison of the amount of water consumed did not reveal striking differences. During the twentieth week females weighing 190 to 200 gm. which received the rice-extract diet with and without fat consumed 27 and 28 cc. of water daily; those fed the yeast diet consumed 26 and 30 cc., respectively; and stock rats weighing 221 gm. consumed 30 cc. However, 28 weeks later males on the yeast diet consumed 47 cc. of water instead of 36 in spite of a drop in weight from 204 to 176 gm.

The scaliness of the hind paws and tail increased with the continued feeding of the yeast diet. At 48 weeks the hind paws were extremely scaly and somewhat erythematous; the tail was very scaly and ringed; the ears were thickened, and the testes had atrophied. Supplements of pyridoxine, pantothenic acid, or rice bran concentrate did not alleviate the symptoms. The animals became scrawny and listless and most of them died. At autopsy, kidney stones were found in 20% of them. Yet at 62 weeks four of the original fourteen male rats were still alive. Two of these had hematuria. Rice bran concentrate did not correct this but the hematuria did disappear after the administration of cottonseed oil.

Fat analyses

Fat analyses were made on the females and on all young which had reached a weight of 40 gm. The animals were killed with ether and analyzed according to the procedure described by Quackenbush and Steenbock (in press). The liver and carcass were analyzed separately (table 4).

The animals on the yeast diet contained less fat than those on the rice-extract diet, but the iodine number was higher.

However, the trend of results after feeding individual fatty acids was the same as on the rice-extract diet. The group receiving ethyl linolenate did not differ in fat content nor in iodine value from those which had received the essential fatty acids. Although unsaturated fat was evidently synthesized by the animals which did not receive dietary fat, and also by those which received ethyl linolenate, it was apparently not of an essential character.

TABLE 4

Analyses of body fats of adult female rats.

GROUP	SUPPLEMENT	RICE-EXTRACT DIET			YEAST DIET		
		Mean body weight	Total fat	Iodine number	Mean body weight	Total fat	Iodine number
		gm.	%		*gm.*	%	
1	No fat	202	11.0	67	186	...	..
2	Cottonseed oil	213	10.8	69	169	8.9	74
3	Ethyl linolate	196	10.7	66	201	7.6	75
4	Ethyl linolenate	192	10.3	70	196	8.3	72
5	Ethyl arachidonate	204	11.4	65	185	8.1	70
7	Ethyl linolate from 2nd week	208	10.4	68	...	...	..

The analyses of the body fats and liver fats of the young produced on the two diets likewise revealed no significant differences. The body fats ranged from 4.8 to 7.1% with iodine numbers from 60 to 78, and liver fats from 1.3 to 3.9% with iodine numbers from 77 to 112. The higher iodine numbers were found in the groups of the lower fat content.

DISCUSSION

The rice-extract diet apparently was superior to the yeast diet because it gave the better growth after the addition of essential fatty acids. This is not inconsistent with the observation of Schneider et al. ('40). However, in view of the results obtained in our long-term experiments, the interpretation of Schneider et al. ('40), that rice bran concentrate can replace the essential fatty acids in all their functions, is not sub-

stantiated. Rice bran concentrate can prevent acrodynia but it cannot prevent the scaliness of the feet and tail.

On the basal diets, failure of reproduction always ensued. After a prolonged gestation period, parturition was accompanied by excessive vaginal bleeding and loss in weight. These results are in agreement with the observations of Maeder ('37) that hemorrhages in the placenta and uterine wall are the cause of fetal death in fat deficiency.

The low percentage and the poor nutritive state of the young weaned in the experiments of others with linoleic supplements were probably due to the low levels fed. Evans et al. ('34) supplemented a yeast diet with 40 mg. of linoleic acid in the form of a concentrate from corn oil. Although this dose was increased to 120 mg. after parturition, only half of the young survived, and those weighed only 28 gm. at weaning. When 25% of lard was included in the diet over 90% survived, and the weight at weaning was 39 gm. Mackenzie, Mackenzie and McCollum ('39) fed 25 mg. of methyl linolate with a diet containing a water-soluble extract of yeast and carried their female rats through three gestation periods. No young were weaned until the second and third gestations and the young of the heaviest litter averaged 31.0 gm. at 21 days. It is possible that the level of methyl linolate was inadequate for the first gestation, but due to a gradual and tenacious storage (Sinclair, '35) sufficient amounts of the essential fatty acids accumulated for the later gestations. In view of these results it is recommended that in reproduction and lactation studies rats should receive daily at least 100 mg. of the essential fatty acids or their equivalent in the form of a natural oil. Turpeinen ('38) has already recommended the use of 100 mg. for maximal growth.

Maeder ('37) has stated that "failure of reproduction in humans due to lack of adequate fat in the diet is considered possible." It is not excluded that reported beneficial effects of wheat germ oil in human pregnancy may have been due in part to meeting the essential fatty acid requirements.

The results of the reproduction studies parallel those obtained when dermal lesions were used as criteria. When assayed with acrodynic rats as described by Quackenbush et al. ('39), 10 mg. daily of either linoleic or arachidonic acid cured the dermal symptoms. As these animals weighed 50 gm. and our females weighed 200 gm., the requirement per kilogram of body weight is about twice as high for reproduction as for the cure of dermal lesions. Linolenic acid was ineffective in both instances. Burr, Brown, Kass and Lundberg ('40) have reported that linolenic acid gave good growth responses but had little effect on the skin. Arachidonic and linoleic acids were similar to each other in their effects. Our data on reproduction as well as on acrodynia have revealed no significant difference of physiological activity between linoleic and arachidonic acids; however, they corroborate abundantly the practical inability of linolenic acid to correct the symptoms of fat deficiency (Hume et al., '38).

CONCLUSIONS

1. On a low-fat diet furnishing only 3.0 mg. of unsaturated lipid or a calculated maximum of 0.8 mg. of linoleic acid per rat per day, rats were raised to maturity and bred. After a prolonged gestation period and severe hemorrhage in parturition, the young were born dead or died soon after birth. A scaly condition of the hind paws and tail was observed after about 10 weeks on the diet.

2. Ethyl linolate and ethyl arachidonate prevented or cured the dermal symptoms completely and produced normal young which were weaned at the age of 3 weeks with an average body weight of 40 gm. The requirement for these acids appears to be higher than previously estimated.

3. Ethyl linolenate did not make possible the production of normal young; neither did it cure the dermal symptoms.

4. Fat analyses revealed a remarkable constancy in both the percentage of total fat and the iodine values of the fat, irrespective of the dietary supplements.

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Douglas M. Webel

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Education:

Ph.D. Animal Sciences, University of Illinois 1998
Research advisor: David H. Baker
Thesis: Amino acid requirements and amino acid utilization as affected by immunological stress.

M.S. Animal Sciences, University of Illinois 1995
Research advisor: Robert A. Easter
Thesis: Effect of betaine supplementation on growth performance, carcass characteristics and nitrogen retention of finishing pigs.

B.S. Animal Sciences, University of Illinois 1993

Experience:

1998-present **Swine Nutritionist**, United Feeds, Inc., Sheridan, IN. Responsibilities include research and development, technical service and training. Develop concepts and protocols for swine nutrition and production research. Summarize, interpret and implement findings for product development and customer service. Provide technical support and training for sales consultants and customers. Extensively involved in developing business structure and providing training and product development for partnerships in the Philippines and China.

1993-1998 **Graduate Research Assistant**, Department of Animal Sciences, University of Illinois. Conducted swine and poultry nutrition research. Skilled in experimental design, diet formulation, statistical analysis, proximate analysis, cell culture and surgical procedures.

1989-1993 **Undergraduate Research Assistant**, Nonruminant nutrition laboratory, University of Illinois. Assisted graduate students with swine nutrition research.

1993 **Swine Farm Manager**, Animal Reproduction Associates, Cropsey, IL. Managed all aspects of a 250-sow farrow to finish swine operation. Conducted contract research trials for various feed and pharmaceutical companies.

Exhibit C

Computer Skills:

Graphics: Harvard Graphics, Power Point
Statistical: SAS
Spreadsheets: Lotus, Excel, Quattro Pro
Word processing: MS Word, WordPerfect
Modeling and simulation: Stella
Diet formulation: E-Z Form, UFFDA, Brill

Laboratory Techniques:

-Nitrogen analysis (macro-kjeldahl)
-Amino acid analysis
-Data entry and analysis
-Surgical catheter placement
-Bioassay
-Diet preparation
-Bomb calorimetry
-Chicken cecectomy
-Cell culture
-Radioimmunoassay

Honors and Achievements:

National Pork Producers Council Award for Innovation-Applied Research Category (2000)

National Pork Producers Council Award for Innovation-Basic Research Category (1996)

Illinois Pork Council Scholarship (1996)

Chicago Mercantile Exchange Pork Industry Scholarship (1993)

American Feed Ingredients Association Scholarship (1993, 1997)

BASF Corp. "Growth is a Promise" Scholarship (1993)

Dean's List

Honors Graduate

Publications:

Peer-Reviewed Publications:

Wolter, B. F., M. Ellis, S. E. Curtis, E. N. Parr and D. M. Webel. 2000. Group size and floor-space allowance can affect weanling-pig performance. J. Anim. Sci. 78:2062-2067.

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Book Chapters:

Johnson, R. W., J. Escobar and D. M. Webel. 1999. Nutrition and Immunology of Swine. In: A. J. Lewis and L. L. Southern (Eds.) *Swine Nutrition*. Butterworth-Heinemann, Stoneham, MA. (submitted).

Abstracts and Technical Articles:

Wolter, B. F., M. Ellis, S. E. Curtis, E. N. Parr and D. M. Webel. 2000. Effects of group size, floor space, and feeder placement on nursery pig performance. J. Anim. Sci. 78 (Suppl. 1).

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References:

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Rodney W. Johnson, Professor, Department of Animal Sciences, University of Illinois at Urbana-Champaign. Phone: (217) 333-2118.

Stephen K. Webel

United Feeds, Inc.

Director Reproduction "Research and Development. Responsible for coordinating sow and boar research and providing technical support to sales consultants.

Purina Mills, Inc.
Bloomington, Illinois

Swine Business Manager. Responsible for the development and implementation of market risk programs and networking concepts in marketing and swine production. Liaison with academic and swine industry leaders. Develop training and information delivery programs for sales and technical staff. Provide consultation to swine producers in reproduction and swine management. Interpret and deliver technical information to sales staff and producers. Prepare and deliver technical presentations at numerous sales and producer meetings. Develop technical manuals and brochures for risk management, networking, and business development programs. Participate in development of business strategies to foster sustainability of producers in Illinois, Iowa, and Missouri.

Farm Owner

Owned and operated a livestock and grain farm.

Illinois State University
Normal, Illinois

Professor of Animal Science. This position was a nine month 100% teaching appointment with courses in swine management, horse science, reproductive physiology, meat science, and introductory animal science. Served as advisor to several student organizations and conducted research programs in reproductive physiology and swine management. Developed innovative interdisciplinary research programs in livestock waste management and odor control.

Animal Production Associates
Cropsey, Illinois

Consultant. Conducted and coordinated research development and marketing projects for commercial firms both domestically and internationally. From 1978 to 1984 coordinated the U.S. development of reproductive control products and provided consultation on international development for Roussel-Uclaf. Developed and reviewed research proposals and coordinated grant funding. Products developed for commercial use include Regumate (Hoechst-Roussel) for estrous cycle control in the horse and pig, PG-600 (Intervet) for stimulation of estrus in gilts and sows, Lutalyse (Upjohn) for induction of parturition in sows.

Abbott Laboratories
North Chicago, Illinois

Senior Physiologist. Responsibilities included planning and coordinating research and pharmaceutical product development for domestic animals in the U.S. and internationally. Products developed for commercial use include PRID (Progesterone

Releasing Intravaginal Device) for estrous control in cattle, Sil Estrus for estrous control in sheep, and GnRH for treatment of cystic ovaries in dairy cattle.

EDUCATION

University of Illinois	B.S. Agricultural Science	1967
Iowa State University	M.S. Animal Science	1969
University of Illinois	Ph.D. Animal Science	1972

PROFESSIONAL ORGANIZATIONS AND RECOGNITION

American Society of Animal Science
- Member Board of Directors 1994-96
- Treasurer 1995-96

American Society of Animal Science – Foundation Board 1999-2000

Midwestern Section American Society of Animal Science
- President-Elect, President, Past-President 1993-96

International Conference on Pig Reproduction
- Organizing Committee and Treasurer 1984-1998

Illinois Council for Food and Agriculture Research (C-FAR)
- Board of Directors, Vice Chair 1994-96

Illinois Governor Livestock Industry Task Force Member 1995-98

Illinois Livestock Industry Activities
- Vision2000 committee and Team2000 committee 1997-98
- Pork Industry Vision Conference Organizing Committee 1998
- Illinois Coalition for Animal Agriculture Planning Committee 1997-98

Flax Meal-Analyzed by ESCL, University of Missouri

Fat by acid hydrolysis	35.46%

Fatty Acid Profile (Expressed as Percent of Total Fat)	
Myristic (14.0)	0.05
Myristoleic (14:1)	0.00
(C15:0)	0.03
Palmitic (16:0)	5.63
Palmitoleic (16:1)	0.08
(17:0)	0.06
(17:1)	0.04
Stearic (18:0)	3.22
Elaidic (18:1t)	0.00
Oleic (18:1)	19.25
Linoleic (18:2)	15.53
Linolenic(Ω18:3)	**54.34**
(Ω18:4)	**0.00**
Arachidic (20:0)	0.12
(20:1)	0.00
(Ω20:3)	**0.00**
Arachidonic (20:4)	0.00
(Ω20:5;EPA)	**0.00**
Docosanoic (22:0)	0.19
Erucic (22:1)	0.05
(Ω22:5)	**0.00**
(Ω22:6;DHA)	**0.00**
Lignoceric (24:1)	0.16
Nervonic (24.1)	0.00

All data presened on an 'as is' basis.
Omega 3 fatty acids (Ω-3) are bolded

	FERTILIUM™	Flax Meal
% Total Fat	20.79%	35.46%
Total Omega 3 Fatty Acid Levels as % of Fat	30.74	54.34
% in Final Product (as is)	0.0639	0.1927

Exhibit E

Dr. Donald E. Orr, Jr.
President & Chief Operating Officer
United Feeds, Inc.
Sheridan, Indiana 46069 USA

- Responsible for overseeing company's feed premix operations, seven feed plants with sales serving twelve Midwest U.S. states, joint venture premix plant in China, three swine research farms and United Feeds Swine Record System. Elected President & COO of United Feeds in 1997.

- Joined United Feeds in 1984 as Vice President of Nutrition and Development, in charge of swine nutrition research and development. Continues as head of R & D program. Since 1993, Don has led United Feeds marketing program in Asia.

- From 1975 to 1984, Don was Assistant and Associate Professor of Animal Science at Texas Tech University, serving as Director of its Swine Research Center. He served as Swine Nutritionist for Central Soya Company from 1974-75.

- Degrees:
  B.S., Purdue University, 1967 (Animal Science)
  M.S., Penn State University, 1969 (Animal Industry)
  Ph.D., Michigan State University, 1975 (Swine Nutrition)

- Memberships professional organizations:
  American Society of Animal Science, Board of Directors
  American Registry of Professional Animal Scientists
  Charter Diplomate, American College of Animal Nutrition
  American Feed Industry Association, Board of Directors
  National Feed Ingredients Association, Board of Directors
  British Society of Animal Science
  Indiana FFA Foundation Board
  Dean's Advisory Council, Purdue University School of Agriculture

- Awards received:
  Distinguished Agricultural Alumnus, Purdue University School of Agriculture, 1999
  "Old Master", Purdue University, 1999

Exhibit F